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FIGURE 1

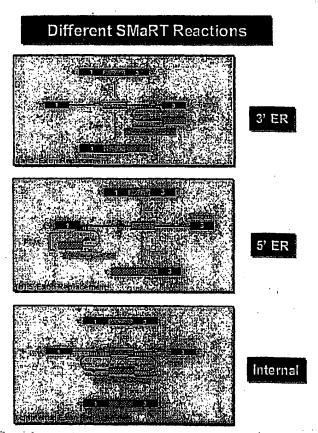


Figure 1. Schematic representation of different SMaRT reactions. (a) Trans-splicing reactions between the target 5' splice site and PTMs 3' splice site, (b) Trans-splicing reactions between the target 3' splice site and PTMs 5' splice site and (c) Replacement of internal exon by double trans-splicing reaction in which the PTM carry both 3' and 5' splice sites. BD, binding domain; BP, branchppoint sequence; PPT, polypyrimidine tract and ss, splice sites.

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2) BPAG2 Target 2 (T2)

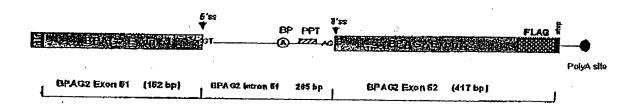


FIGURE 2B

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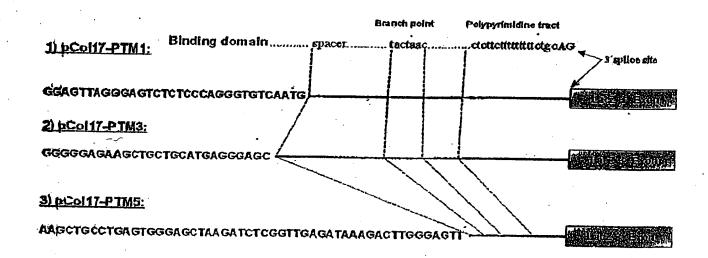


FIGURE 2C

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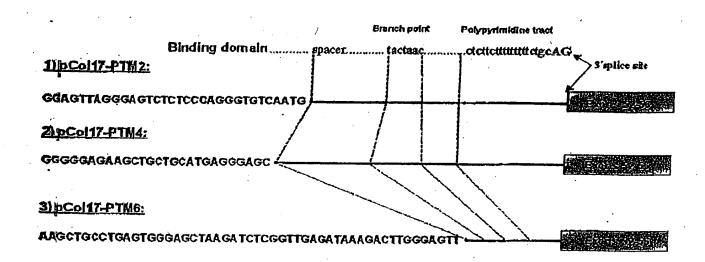
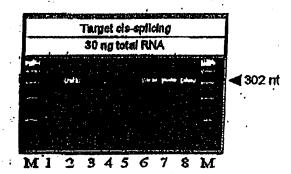
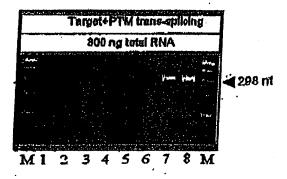


FIGURE 2D

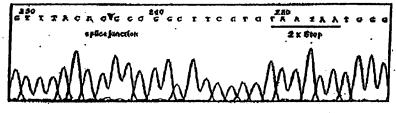
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sequence desplicing



sequence trans-splicing

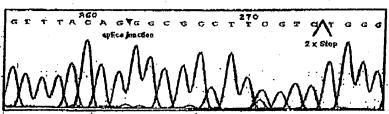


FIGURE 3A-B

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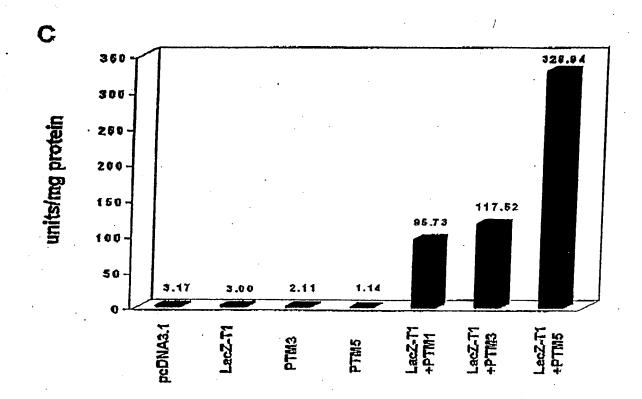


FIGURE 3C

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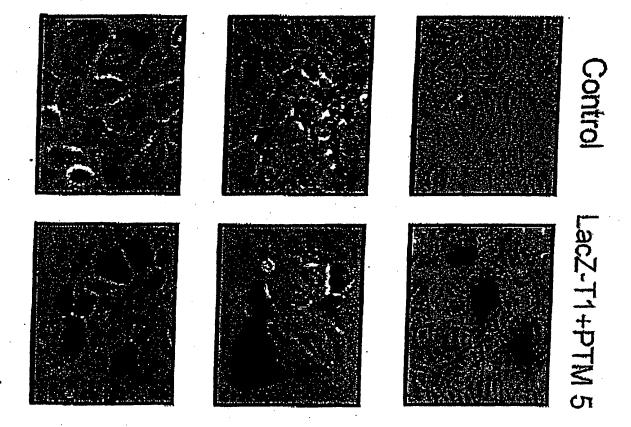
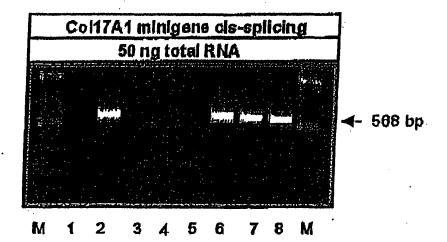


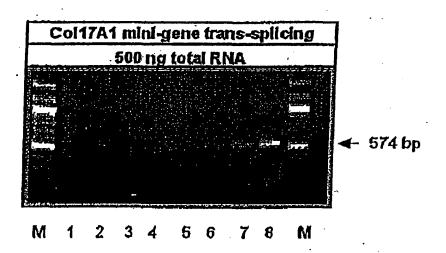
FIGURE 4

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COL17A1 minigene (T2)



FIGURE 5A-B

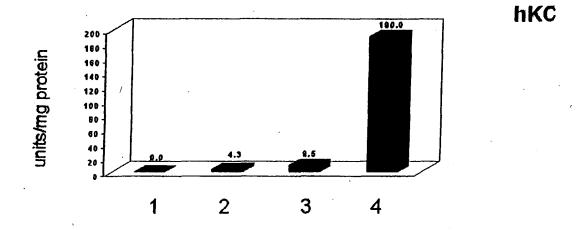
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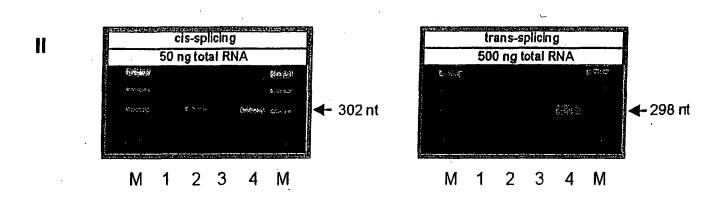


FIGURE 6A

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295.6 List 100 0.0 8.0 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150 0.0 150

GABEB



11

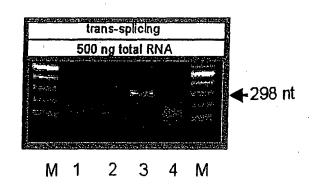
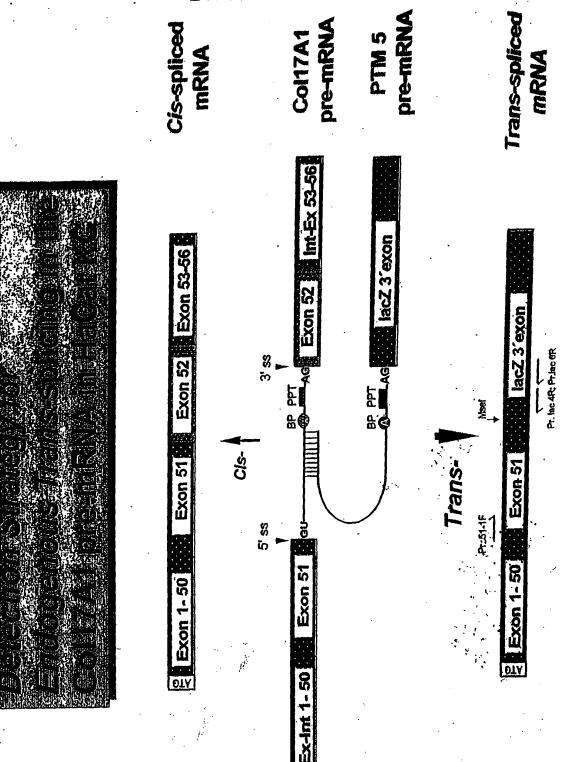


FIGURE 6B

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FIGURE 7

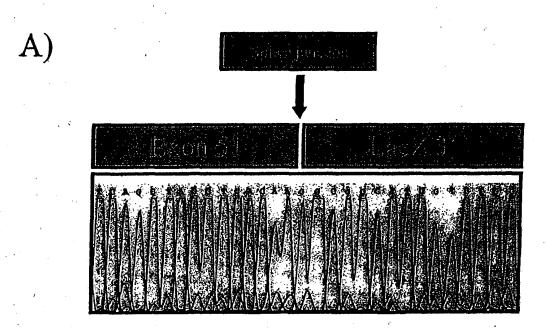


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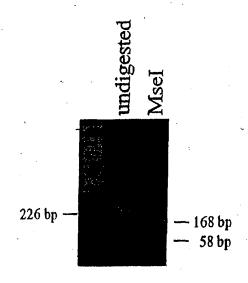
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FIGURE 8

Endogenous Transsplicing of Col17 pre-mRNA with PTM5 pre-mRNA



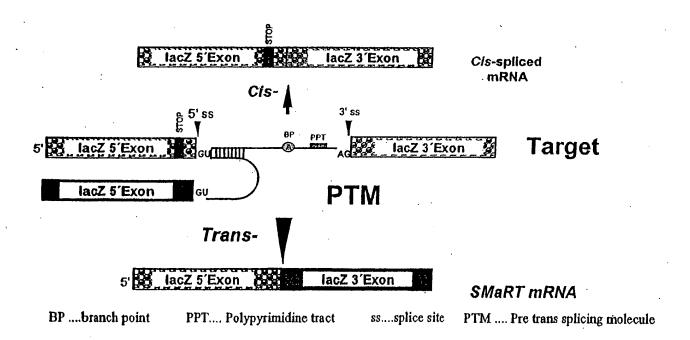
B)



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FIGURE 9

5' trans-splicing in a LacZ-model system for hereditary diseases

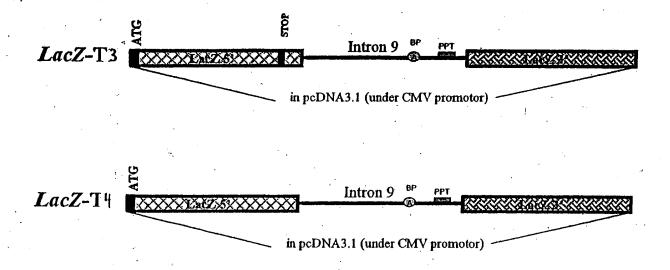


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FIGURE 10

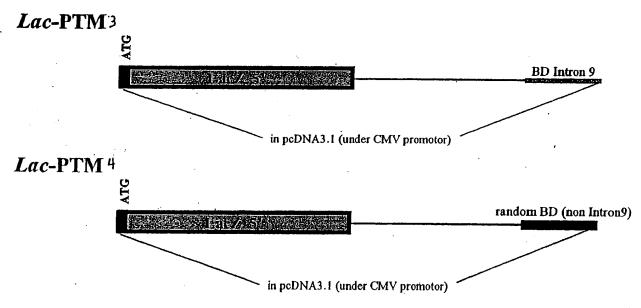
Target LacZ-T3 (containing Intron 9) and LacZ-T4 used for optimizing trans-splicing and transfection conditions



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FIGURE 11

LacZ-PTM-3 (Intron 9 specific binding domain) and LacZ-PTM-4 (non specific binding domain) for establishing optimal transsplicing conditions



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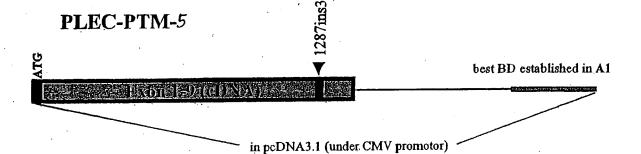
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FIGURE 12

PLEC-PTM-5 for the introduction of the 1287ins3 mutation in 293T cells

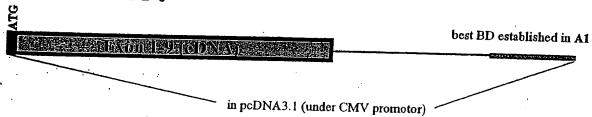


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FIGURE 13

PLEC-PTM-6 for the repair of the 1287ins3 mutation in plectin deficient patient cells

PLEC-PTM-6

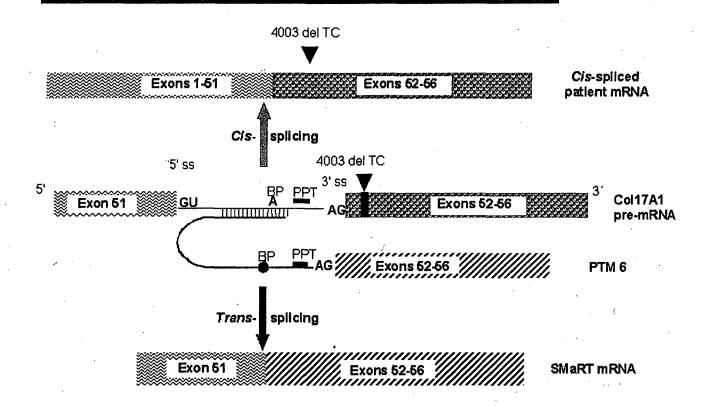


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FIGURE 14A

Trans-splicing Strategy for Col17A1 Gene (GABEB/generalized atrophic benign epidermolysis bullosa)



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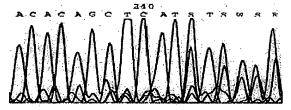
FIGURE 14B

Semi-nested RT-PCR with BPAG2primer 51-1F, 53-1R and 52-1R

RT-PCR









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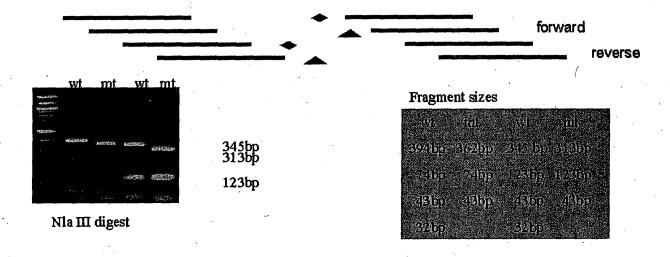
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394bp 362bp

74bp

FIGURE 14C

TOPO Cloning + Nla III digest



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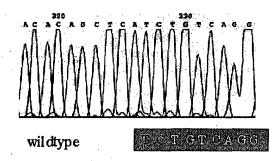
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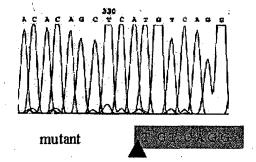
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FIGURE 14D

Sequencing of mutant and wildtype clones confirmed the correct *trans*-splicing of PTM6 into GABEB cells





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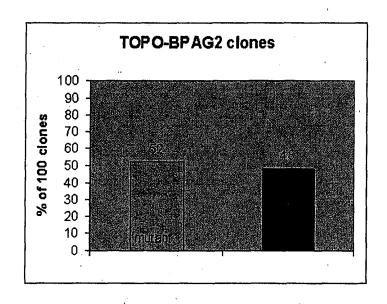
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FIGURE 14E

Analysis of 100 clones



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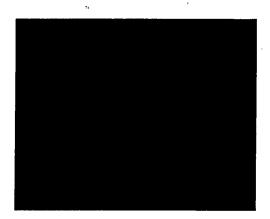
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FIGURE 15

Immunofluorescence of PTM6transfected GABEB cells

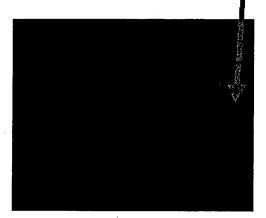
GABEB

non-transfected



GABEB

PTM6 transfected, 3 days



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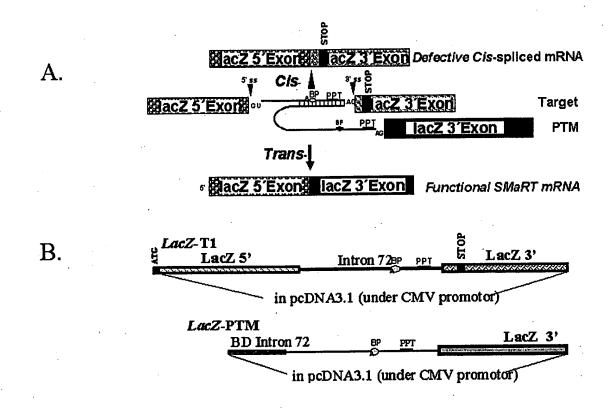
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FIGURE 16A-B

Trans-splicing strategy for COL7A1 gene (Dystrophic epidermolysis bullosa)

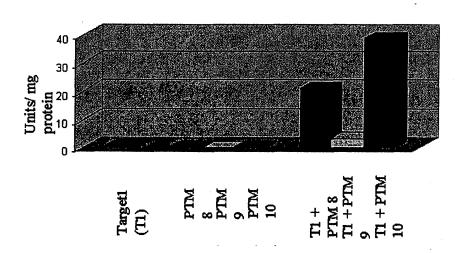


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FIGURE 17

B-gal Assay of a Cotransfection of Col7 Target T1 and PTM 8, 9, 10 in 293T cells

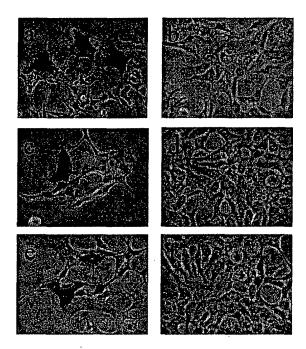


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FIGURE 18

Beta-gal staining of Col7 PTM transfected human 293T cells



left panel: (a) Target + PTM8, (c) Target + PTM9, (e) Target + PTM10; right panel: corresponding control PTM without Target.